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Monoclonal Antibody Form and Function: Manufacturing the Right Antibodies for Treating Drug Abuse

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ABSTRACT

Drug abuse continues to be a major national and worldwide problem, and effective treatment strategies are badly needed. Antibodies are promising therapies for the treatment of medical problems caused by drug abuse, with several candidates in preclinical and early clinical trials. Monoclonal antibodies can be designed that have customized affinity and specificity against drugs of abuse, and because antibodies can be designed in various forms, in vivo pharmacokinetic characteristics can be tailored to suit specific clinical applications (eg, long-acting for relapse prevention, or short-acting for overdose). Passive immunization with antibodies against drugs of abuse has several advantages over active immunization, but because large doses of monoclonal antibodies may be needed for each patient, efficient antibody production technology is essential. In this minireview we discuss some of the antibody forms that may be effective clinical treatments for drug abuse, as well as several current and emerging production systems that could bridge the gap from discovery to patient use.

KEYWORDS: antibody therapy, antibody production

INTRODUCTION

Antibody treatments are in preclinical and clinical development for addressing a wide range of medical problems caused by drug abuse. These treatments include vaccination to help prevent relapse to nicotine addiction,[1,2] and monoclonal antibodies (mAb) to (1) treat overdose from phencyclidine or methamphetamine,[3-5] or (2) prevent relapse to methamphetamine abuse.[6]

A considerable obstacle for mAb therapy (eg, immunoglobulin G [IgG], antigen binding fragment [Fab]; single chain antigen binding fragment [scFv]; Figure 1) is that discovering potentially important mAb medications and then producing sufficient quantities for rigorous preclinical or clinical testing takes a while. This is a major hurdle that must be overcome if this new technology is to be taken to clinics in a timely manner. Because the early-stage manufacturing and formulation of mAb-based medications is both technologically challenging and expensive, this review will examine the production of these therapies at the levels needed for preclinical in vivo studies (eg, 0.5-10 g), with a special emphasis on alternative production systems, including plants.

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POTENTIAL ADVANTAGES OF MAB THERAPY

Passive immunotherapy with mAb has important advantages over active immunization. First, significantly larger doses of mAb can be administered, and protection is immediate. Second, the duration of action of mAb is more predictable than antibody generated by active immunization and likely to be related to the biological half-life of the mAb. For example, currently approved mAb medications for treating other disease processes (eg, cancer) have half-lives of up to 28 days in humans.[7] Third, unlike with active immunizations, there is no immunological memory of the abused drug and the possibility of unexpected cross reactivity with endogenous ligands is less likely with mAb.

The frequency of mAb dosing should be based on the fundamental principles of clinical pharmacokinetics. For instance, dosing with mAb could occur once every mAb half-life, and loading dosing could be easily predicted for use in achieving rapid steady-state mAb concentrations. It is also important that administration of mAb could provide immediate protection to patients at critical times in the addictive disease process, when drug craving is high and relapse may be imminent. Rapid clinical effects would also be needed when using the mAb medications to treat drug overdose.

Another advantage of mAb medications is the ability to preselect for the affinity and specificity of the antibody, and thereby have the medication parameters be constant from one production lot to the next. The ideal specificity and

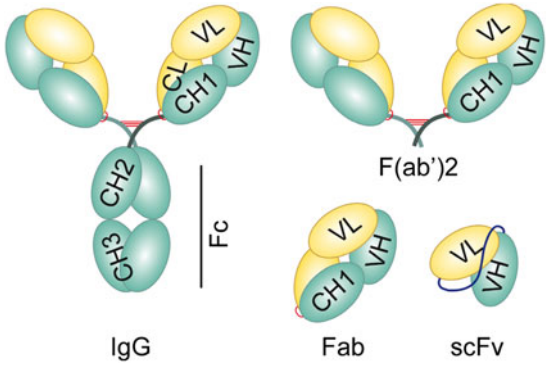

Figure 1. Selected antibody forms relative to therapeutic applications. Each oval represents an immunoglobulin folding domain. Disulfide bonds are represented as red lines, and the polypeptide linker of the scFv is represented by a blue connecting ribbon. VL indicates variable domain light chain; VH, variable domain heavy chain; CL, constant domain light chain; CH, constant domain heavy chain; Fc, Fc fusion; IgG, immunoglobulin G; F(ab')2, dimeric antigen binding fragment; Fab, antigen binding fragment; scFv, single chain antigen binding fragment.

affinity of each antibody can be selected for the intended purpose. For example, an antimethamphetamine antibody should be highly specific for the abused (+)-methamphetamine isomer, while having little affinity for (–)-methamphetamine, which is commonly used in over-the-counter inhalants, or endogenous molecules.

It is also possible to use the same mAb to produce other functional isoforms for treatment of other drug abuse–related medical applications. For instance, an IgG would be best suited for medical situations where a long duration of action is needed (eg, relapse prevention). Human IgG1, IgG2, and IgG4 have extended half-lives because of catabolic protection that is afforded by the so-called neonatal receptor (FcRn).[8,9] Human IgG3 has a much shorter half-life compared with the other human IgG isoforms because of a single amino acid difference in the FcRn binding domain[10]: at position 435 there is an arginine instead of a histidine.

Small antidrug binding fragments could also prove medically useful. The same IgG used for preventing relapse to drug abuse could be converted to a Fab or scFv. This would reduce the molecular weight of the protein from ~150 000 Da to ~50 000 Da for Fab and 27 000 Da for scFv. Since both Fab and scFv would be derived from the binding sites of the intact IgG, there would be no significant loss in affinity or specificity. The major change would be in the pharmacokinetic properties and routes of elimination. Instead of having a half-life of ~3 weeks for the IgG, the Fab and scFv half-life would be less than 1 day (eg, 0.5-21 hours),[7,11] because of rapid clearance of Fab and scFv by renal glomerular filtration.

Potential Disadvantages of mAb Therapy

Antibody-based therapies could potentially save lives and reduce the crippling effects of chronic drug abuse, but they will not be a magic bullet to cure addiction. In many ways the problems associated with treating drug abuse are analogous to the problems associated with treating a chronic infectious disease (eg, HIV). For instance, certain individuals and segments of the population are more susceptible to infectious diseases, and the best way to prevent epidemics is to stop the spread of the disease from individual to individual. By analogy, we need medical prevention to aid individuals and groups who are at an increased risk for addiction, and we need specific medications for treating key individuals who are important vectors for spreading drug abuse in the population.

Although mAb have significant promise as therapeutic agents, they are not without problems. The 3 major problems are the high cost, the risk of toxicity, and the potential for allergic-type reactions. The current cost for mAb medications for treating cancer and other health problems is thousands of dollars per month. Although this is a great deal of money, it is still significantly less than the cost of a day in a hospital emergency room or intensive care unit, or even time in prison, and the accompanying costs to society. Ideally, the cost of antibody production will continue to decrease with advances in technology.

Toxicity may also result from the expected pharmacological effects of the antibody treatment of drugs of abuse. For instance, if a patient is addicted to a drug like methamphetamine, rapid removal of the drug by the mAb could precipitate withdrawal, with subsequent clinical manifestations. Nonspecific toxicity may occur, including infusion reactions, cytokine release, and hypersensitivity to foreign immunoglobulins. Toxicity after treatment with mAb may be anticipated and prevented in some cases based on the pretesting of the mAb in immunohistochemical studies using human tissues. The US Food and Drug Administration requires this type of testing before human clinical trials begin.

Hypersensitivity reactions to the xenogeneic component of chimeric and humanized antibodies can occur upon the first dose of antibody and following repeated exposure. Development of an immune response to the mAb medication can adversely affect the antibody's pharmacokinetic, safety, and efficacy profile. All therapeutic antibodies approved to date have shown some degree of immunogenicity, even in immunosuppressed patients.[7] An antiglobulin response to an mAb medication may be anti-isotypic, anti-idiotypic, or anti-allotypic. An anti-isotypic response is directed against the constant regions of the heavy or light chains and may not directly impair antigen binding, although acceleration of mAb clearance through immune complexation may cause

mAb serum concentrations to drop below effective levels. Anti-idiotypic antibodies develop against idiotypes of the mAb variable region and can block the antigen binding site in addition to accelerating mAb clearance. Anti-allotypic responses may occur in individuals who are homozygous for polymorphisms of the IgG constant regions. mAb immunogenicity may also be enhanced by aggregates in the dosing formulation or by posttranslational modifications, such as glycosylation, that may present antigenic determinants.

Antiglobulin responses to antibody products usually alter pharmacokinetic profiles. Immune complex formation accelerates clearance, reduces serum levels, and impairs targeting of the therapeutic antibodies.[7,12] Serious safety risks may be associated with immunogenicity. Adverse reactions may be local or systemic and may vary from mild injection site reactions to life-threatening anaphylaxis.[7,13] Accelerated clearance of therapeutic antibodies or neutralization of the antigen binding domain can result in loss of product efficacy, impaired antigen targeting, or interference with antibody-based diagnostic tests.[7,13,14]

Production Systems for mAb and Different mAb Forms

While different mAb forms (IgG, Fab, scFv) could provide distinct therapeutic advantages for the treatment of specific medical needs such as overdose or relapse, producing each of these different mAb forms in gram quantities suitable to conduct both in vitro and in vivo preclinical studies is extremely challenging, especially within a reasonable time frame (6 months or less). Each mAb and mAb form is a unique protein with distinct folding requirements as well as unique biochemical and biophysical characteristics that affect its ability to be expressed at high levels as a soluble, biologically active protein. To date, no single expression system appears ideal for the production of mAb or engineered mAb fragments. Even the rapid production of recombinant full-length mAb on an intermediate scale by traditional mammalian cell culture can be problematic because of the length of time (~1 year) required to identify and establish a high-producing stably transformed cell line.

To address time and protein production level requirements, alternative production systems or production strategies are being developed. These techniques show promise for the generation of mAb and mAb fragments at the intermediate levels needed to conduct preclinical studies. In addition, many of these approaches can then be scaled or adapted for eventual use in clinical applications. Alternative production systems include bacteria, yeast, insect cells, plants, and animals, including, most recently, eggs from chimeric chickens.

The use of plants or animals may hold little advantage over traditional mammalian cell culture for intermediate-level production. This is because of the amount of time required to establish a high-producing transgenic line (1 year or longer) and the diverse technical demands associated with adapting this technology. However, new transformation technologies are both reducing these time lines and simplifying the methods required to take advantage of plants and cultured mammalian cells for intermediate-level mAb production.

Production of scFv and Fab Antibody Fragments

Mammalian Cell Expression

While transgenic mammalian cells (eg, Chinese hamster ovary cells [CHO]) grown in culture are the industry standard for producing full-length mAb, work to date indicates that mammalian cells are less suited for high-level production of scFv, despite the less complex structure of these engineered fragments. Typical expression levels for an scFv in mammalian cells are on the order of 1 to 4 mg/L, which is tens to hundreds of times below the level of mAb achieved using mammalian cells.[15] Consequently, scFv production has been explored in prokaryotic and other eukaryotic expression systems.

Escherichia coli Expression

Results of these studies indicate that bacteria are well suited for accumulating properly folded scFv. Although bacteria lack a compartmentalized secretory pathway that in mammalian cells is required for proper folding, disulfide bond formation, and glycosylation, bacteria do possess the necessary protein machinery to promote disulfide bond formation. The disulfide bond proteins (or dsb proteins), which function in the periplasmic space surrounding the bacterial cytoplasm, promote efficient formation of disulfide bonds and the isomerization of incorrectly formed disulfide bonds.[16] Even scFv, which contains far fewer disulfide bonds than mAb and no interchain disulfide bonds, possesses an intrachain disulfide in each variable domain that must form for proper function.

Given the successful production of scFv in the bacterial periplasm, mAb production has also been attempted. Results of these studies indicate that multiple mAb assembly intermediates accumulate in the periplasm, with only a small percentage of recombinant protein representing fully assembled IgG.[17] Fab fragments, which are less complex than mAb but require the formation of a single interchain disulfide, accumulate to levels between what is observed for scFv and mAb. Whereas scFv accumulation as high as 100 to 130 mg/L of culture can be achieved (refolded from inclusion bodies), Fab levels are not typically greater than 10 to 30 mg/L.[18]

We have examined the ability of *Escherichia coli* to express both scFv and Fab fragments. For Fab production, both antimethamphetamine and antiphencyclidine mAb were cloned into dual expression plasmids suitable for simultaneous expression of the light chain (LC) and corresponding heavy chain (HC) Fab domain (Fd). When a Strep affinity tag was placed at the C-terminus of the LC and a 6-histidine tag was placed at the C-terminus of the HC Fd, it was possible to select for fully assembled Fab using sequential affinity chromatography steps. Purified antimethamphetamine and antiphencyclidine Fab exhibit the correct size (~50 kDa), lack detectable bacterial contaminants, and possess the same affinity as the parent mAb (Figure 2). It should be noted that both the level of expression and the solubility of *E coli*–expressed scFv and Fab can vary greatly. Accumulation of soluble Fab required expression at 21°C. We found that higher growth temperatures limited the accumulation of soluble Fab and favored the formation of insoluble inclusion bodies. In addition, a similar antimethamphetamine scFv expressed in *E coli* formed insoluble inclusion bodies regardless of culture conditions examined and required protein refolding to acquire antigen binding properties.

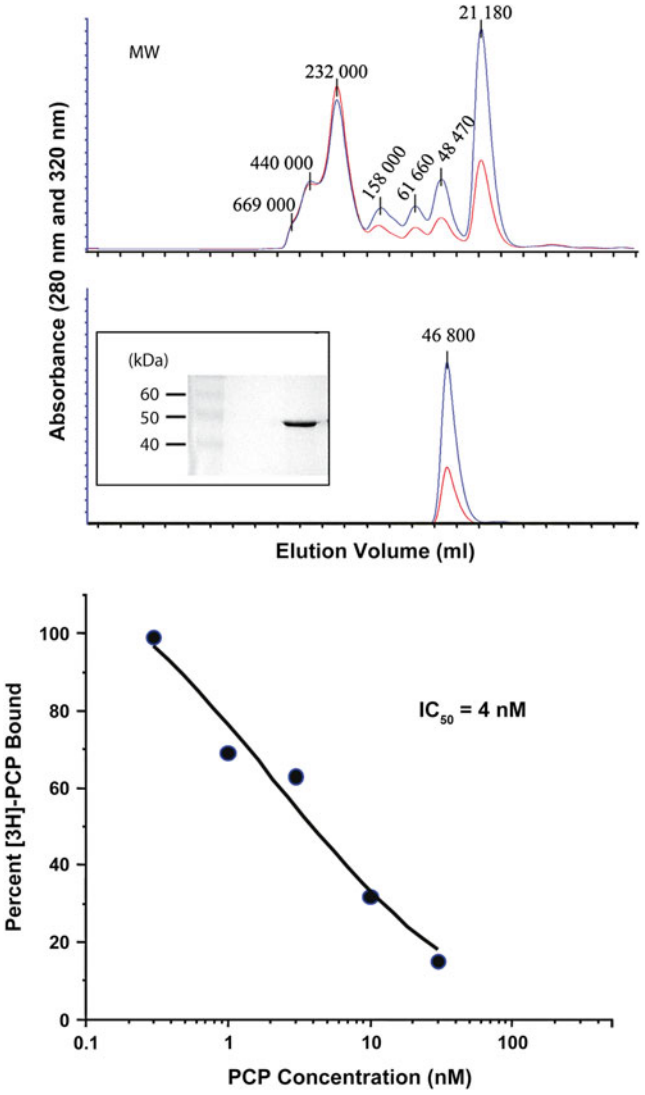

Figure 2. Recombinant anti-PCP Fab produced in *Escherichia coli* has the same affinity as the parent mAb produced in mammalian cell culture. Left: Anti-PCP Fab purified from *E coli* assembles into a 46 800-Da heterodimer as judged by sizing chromatography and nonreducing PAGE (inset). Right: Binding of recombinant anti-PCP Fab to radiolabeled PCP was conducted in the presence of increasing unlabeled PCP. The concentration of PCP required to produce a 50% inhibition of [^{3}H]-PCP binding (IC_{50}) mimics the binding characteristics of the parent mAb. PCP indicates phencyclidine; Fab, antigen binding fragment; mAb, monoclonal antibodies; PAGE, polyacrylamide gel electrophoresis; MW, molecular weight; IC, inhibitory concentration.

Yeast Expression

We have found that scFv insolubility can be overcome by expression in the methylotrophic yeast *Pichia pastoris*[15] (see Cereghino et al[19] for review), using the yeast's traditional eukaryotic secretory system to properly fold and export scFv into the culture media. This folding pathway employs the use of conserved chaperone and disulfide bond–forming proteins found in the *Pichia* endoplasmic reticulum that are absent in prokaryotes. Importantly, the affinity and specificity of scFv expressed in yeast appears identical to the parent mAb's.[20] Consistent with our observations, yeast-based expression provides high levels of correctly folded scFv (and Fab), which is recoverable in a single chromatographic step through the use of small affinity tags (eg, 6 histidine; Figure 3). *P pastoris* is capable of extremely high cell densities in bioreactor cultures (≥400 g/L fresh cell weight), and protein levels on the order of 0.02 to 4 g/L of culture are reported.[21] An additional advantage of the *P pastoris* expression system is the ability to express therapeutic proteins in an organism that is free of animal viruses and endotoxin contamination.[22]

A potential drawback with the use of yeast-based expression systems compared with bacteria production is the added time required to develop cell lines with suitable expression levels (2-3 months). Unlike *E coli*–based systems, where expression is driven from complementary DNA (cDNA) harbored within replicating plasmids, yeast-based expression systems rely on genomic insertion of cDNA, which creates expression variability between cell lines. This is because expression levels are sensitive to the site of chromosomal insertion and the number of cDNA insertion events. Hence, yeast-based expression requires screening of different transformed cell lines in order to identify a cell line expressing suitable levels of a recombinant mAb fragment. For this reason, yeast-based expression is often explored only after attempting production in *E coli*. In some cases, antibody fragment–specific problems can be incurred using yeast-based expression systems—most notably, sensitivity to secreted proteases or the presence of a

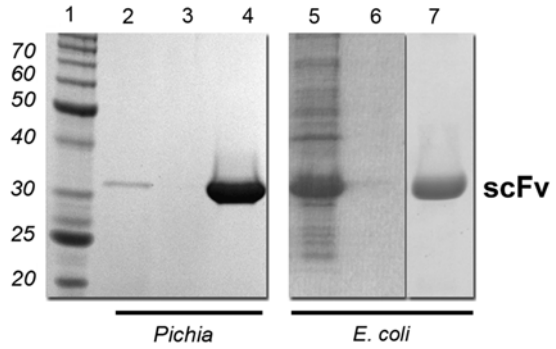

Figure 3. scFv production in *Pichia pastoris* produced as a soluble protein that can be purified in 1 chromatography step. Coomassie-stained SDS-PAGE of 6-His tagged scFv produced in *Pichia* and *Escherichia coli* cells. Lane 1, MW marker. Lane 2, unclarified media from *Pichia* bioreactor run after 72 hours of production. Lane 3, flow through fraction from nickel-affinity chromatography column. Lane 4, elution of scFv from column. Lane 5, total cell lysate from the same scFv expressed in *E coli* after 18 hours. Lane 6, soluble fraction from *E coli* lysate. Lane 7, scFv after isolation from inclusion bodies, refolding, and nickel-affinity chromatography. scFv, single chain antigen binding fragment; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; MW, molecular weight.

nonconserved glycosylation site in the HC variable region. This can lead to the addition of highly antigenic mannose-rich structures unique to yeast. However, proteolytic sensitivity can be circumvented using altered culture conditions,[23] and most scFv and Fab fragments lack glycosylation sites and are produced as aglycosylated proteins, as is done in *E coli*–based systems. In applications such as treatment of drug abuse with mAb-based medications, where glycosylation is not required to activate cell-based immunity, yeast could provide a suitable alternative to the production of full-length therapeutic mAb. This would require the replacement of Asn 297 in the HC with Ala or Gln to eliminate the conserved N-linked glycosylation site found in the Fc domain, which is not a prerequisite for proper antibody folding.

Plants as Antibody Factories

A large number of studies have established that plants can efficiently produce high levels of scFv or Fab,[24] partly because of the conserved nature of the secretory pathway in plants and animals. The pathway provides the proper folding and disulfide bond formation needed to assemble functional mAb fragments. Plant-based production systems possess several advantages over traditional mammalian-based production systems, including the lack of endotoxins and other potentially harmful agents (eg, prions, viruses). The scalability of plant production systems and the low cost associated with plant culture, compared with establishing and maintaining industrial-scale cell (bacterial, yeast, or mammalian) culture facilities, make plants an attractive production system for mAb-based therapies. Yet the time investment and technology needed to develop transgenic plant lines suitable for intermediate protein production levels have limited the adoption of this novel production system at the preclinical and clinical medications development level. This could well change with the advent of recently developed second-generation expression technology.

New expression technology in plants uses Agrobacterium to efficiently transfer many copies of recombinant DNA into leaf cells of intact plants. The transfer DNA, which contains the coding sequence and all of the elements for recombinant protein expression, supports transient expression of target protein in the leaf cells, eliminating the need for time-intensive tissue culture–based transformation methods. However, high levels of recombinant protein production come from coupling transient expression technology with the coexpression of viral replicons, which amplify the coding information for the recombinant target protein.[25-28] Used together, these technologies have led to levels of cytosolically expressed green fluorescent protein as high as 2.5 to 4 g/kg of leaf fresh weight in tobacco. This is even more impressive if you consider that a kilogram of leaves can be produced by only a few plants. Importantly, these recombinant protein levels were reached in only 8 days following transient transformation by Agrobacterium. The combined use of transient transfection and viral replicons has been termed magnifection. Neither scFv nor Fab has been reported to have been produced in plants by magnifection technology. However, this technology has been shown to support high-level plant-based production of recombinant secretory proteins. When routed to the secretory pathway in leaf cells of intact tobacco, human growth hormone accumulated in the leaf apoplast at levels that reached a stunning 1 g/kg fresh weight.[29] This provides a strong indication that magnifection or similar technology can be applied to the rapid high-level production of mAb forms required for preclinical or clinical applications since all of these mAb forms must pass through the secretory pathway in plants for proper folding and disulfide bond formation.

PRODUCTION OF RECOMBINANT MAB

Could the same magnifection technology developed for high-level recombinant protein production in plants be applied to the rapid high-level production of full-length mAb in plants? While stably transformed plants have been shown in numerous studies to express and accumulate fully assembled and functional mAb, other factors may limit the immediate usefulness of plants for mAb production. Perhaps the greatest limitation to plant-based mAb production is the apparent high level of postassembly mAb degradation that takes place in plants. This problem appears to be substantially underrepresented in the literature and was once thought to be a protein assembly issue.

More recent data from a tobacco-based expression system indicate that half or more of the expressed mAb is degraded to its antigen binding fragments (mostly F(ab')2; Figure 4).[30] Our own studies, which used tobacco to express a murine IgG1 (anti-phencyclidine mAb6B5), support these findings (Figure 4). The vast majority of mAb6B5 recovered from soluble leaf extract by protein G affinity chromatography is found as 1 of 3 differently sized protein species observed by nonreducing gel electrophoresis (Figure 4, left panel). Each of these proteins is recognized on Western blots with polyclonal antibodies directed against either murine IgG or murine kappa LC. These mAb fragments are observed even when protein extraction is conducted in the protective presence of protein denaturant, indicating that the fragments were formed in the plant prior to extraction and not by proteases released during the extraction process (results not shown). Importantly, the vast majority of plant-produced mAb6B5 is present as a F(ab')2-sized product (~70% of total mAb recovered) that contains an intact LC and an HC fragment lacking most of the Fc region in the HC (Figure 4, right panel). Although it has been suggested that inefficient antibody assembly is a cause of the accumulation of mAb fragments in plants (eg, tobacco), more recent studies point to proteolytic cleavage of fully assembled mAb as the source of mAb fragments. Specifically, treatment of plant cells with an inhibitor of Golgi vesicle formation prevents IgG1 degradation.[30] Since antibodies reaching the Golgi network have already been assembled in the endoplasmic reticulum, proteolytic degradation of HC in plants must take place after antibody assembly, presumably at a step following residence in the Golgi. Given the robust ability of plants to produce engineered antibody fragments (eg, scFv), efforts to successfully eliminate proteolytic degradation of the HC Fc domain could help make plants a universal platform for the production of full-length mAb and engineered mAb fragments.

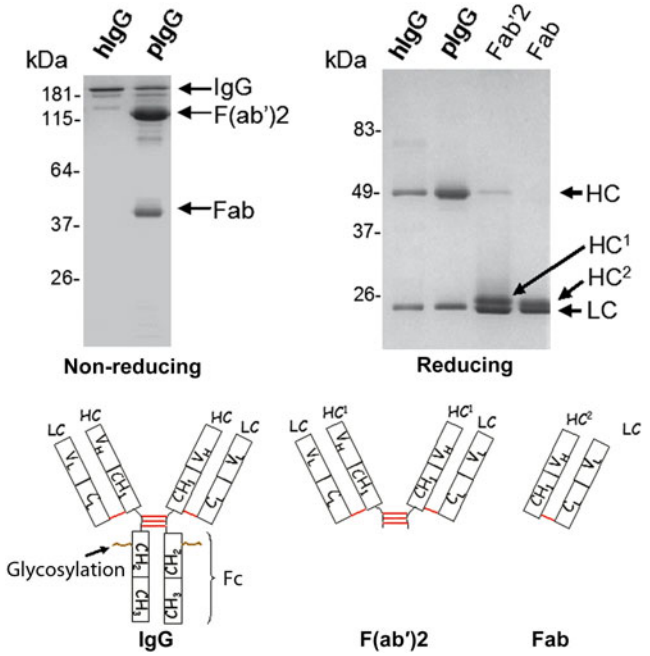

Figure 4. IgG HC is susceptible to proteolytic cleavage in plant-based antibody production systems. Left: mAb6B5 murine IgG1 was recovered from tobacco leaves (pIgG) or cultured hybridoma cells (hIgG) by Protein G affinity chromatography and examined on nonreducing SDS-PAGE. Right: Bands corresponding to IgG, F(ab')2, and Fab were then excised from the gels and reexamined on SDS-PAGE reducing gels to identify individual polypeptides. Lower: Antibody models correspond to the most abundant mAb species identified in tobacco. IgG, immunoglobulin G; HC, heavy chain; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; F(ab')2, dimeric antigen binding fragment; Fab, antigen binding fragment; mAb, monoclonal antibodies; LC, light chain; CH, constant domain heavy chain; CH_1, constant domain 1 of heavy chain; V_H, variable domain of heavy chain; V_L, variable domain of light chain; C_L, constant domain of light chain.

Unlike scFv and other mAb fragments, recombinant mAb can be produced at high levels by mammalian cells. The major drawback of current mammalian cell culture technologies is the time and cost associated with developing a stable mAb-producing cell line for each recombinant mAb. Hence, the technology is not well suited for situations where mAb-based medications that require large individual patient doses are being developed or where multiple renditions of a single mAb are desirable to examine the function of specific mAb features, such as the importance of glycosylation or the role of a specific residue in the binding pocket.

Advances in transient transfection technologies have begun to address the issue of production time and cost. Because of the development of highly efficient transfection reagents, it is now possible for many copies of plasmid DNA carrying the target LC and HC genes to be introduced into a single cell. The plasmid DNA can function in protein expression without needing to be stably integrated into the genome. High levels of protein can be produced in only a few days, and levels of mAb secreted into the media often range between 20 and 40 mg/L of mammalian cell culture.[31,32] Importantly, the process can be scaled to produce gram quantities in considerably less time than it takes to develop a transgenic cell line. However, these innovations in transient transformation of mammalian cells still do not address the financial bottleneck associated with the cost of mammalian cell cultures.

Conclusion

mAb and mAb engineered fragments present potentially effective therapies to treat drug abuse. The high affinity and exquisite specificity of these proteins to their drug targets and their customizable pharmacokinetic properties make them very attractive therapeutic candidates. However, like

many protein-based pharmaceuticals, these medications are difficult to move from the bench to the clinic because of the large amounts needed for therapeutic doses. *E coli*, the prototypic protein production system for many proteins, is not able to produce intact IgG efficiently and often produces too few soluble antibody fragments. Thus, lower eukaryotes such as *P pastoris* are becoming more widely used for antibody fragment production, and the relative simplicity of their genetic manipulation make this system an attractive alternative to *E coli*. However, the potential for proteolytic degradation and unwanted glycosylation, as well as the need for special equipment (bioreactors), must be considered. Plants are emerging as a viable expression system for engineered mAb fragments and have enormous protein production capabilities. However, plant-based systems have until now been technically difficult to genetically modify and are plagued with IgG degradation problems. New advances in molecular technology and our understanding of these complex systems are beginning to overcome these issues and will eventually help stimulate cost-effective protein-based therapies for the treatment of drug abuse, so that these medications can become widely available.

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REFERENCES

1. Pentel P, Malin D. A vaccine for nicotine dependence: targeting the drug rather than the brain. *Respiration (Herrlisheim)*. 2002;69:193-197.

2. Hieda Y, Keyler DE, Ennifar S, Fattom A, Pentel PR. Vaccination against nicotine during continued nicotine administration in rats: immunogenicity of the vaccine and effects on nicotine distribution to brain. *Int J Immunopharmacol*. 2000;22:809-819.

3. McClurkan MB, Valentine JL, Arnold L, Owens SM. Disposition of a monoclonal anti-phencyclidine Fab fragment of immunoglobulin G in rats. *J Pharmacol Exp Ther*. 1993;266:1439-1445.

4. Valentine JL, Mayersohn M, Wessinger WD, Arnold LW, Owens SM. Antiphencyclidine monoclonal Fab fragments reverse phencyclidine-induced behavioral effects and ataxia in rats. *J Pharmacol Exp Ther*. 1996;278:709-716.

5. Byrnes-Blake KA, Laurenzana EM, Carroll FI, et al. Pharmacodynamic mechanisms of monoclonal antibody-based antagonism of (+)-methamphetamine in rats. *Eur J Pharmacol*. 2003;461:119-128.

6. McMillan DE, Hardwick WC, Li M, Owens SM. Pharmacokinetic antagonism of (+)-methamphetamine discrimination by a low-affinity monoclonal anti-methamphetamine antibody. *Behav Pharmacol*. 2002;13:465-473.

7. Roskos LK, Davis CG, Schwab GM. The clinical pharmacology of therapeutic monoclonal antibodies. *Drug Dev Res*. 2004;61:108-120.

8. Brambell FW, Hemmings WA, Morris IG. A theoretical model of gamma-globulin catabolism. *Nature*. 1964;203:1352-1354.

9. Junghans RP. Finally! The Brambell receptor (FcRB). Mediator of transmission of immunity and protection from catabolism for IgG. *Immunol Res*. 1997;16:29-57.

10. Ghetie V, Ward ES. Transcytosis and catabolism of antibody. *Immunol Res*. 2002;25:97-113.

11. Trang J. Pharmacokinetics and metabolism of therapeutic and diagnostic antibodies. In: Ferraiolo B, Mohler M, Gloff C, eds. *Protein Pharmacokinetics and Metabolism.* New York, NY: Plenum Press; 1992:223-270.

12. Weiner LM. Monoclonal antibody therapy of cancer. *Semin Oncol*. 1999;26:43-51.

13. Stein KE. Immunogenicity: concepts/issues/concerns. *Dev Biol (Basel)*. 2002;109:15-23.

14. Kuus-Reichel K, Grauer LS, Karavodin LM, Knott C, Krusemeier M, Kay NE. Will immunogenicity limit the use, efficacy, and future development of therapeutic monoclonal antibodies? *Clin Diagn Lab Immunol*. 1994;1:365-372.

15. King DJ, Byron OD, Mountain A, et al. Expression, purification and characterization of B72.3 Fv fragments. *Biochem J*. 1993;290:723-729.

16. Kadokura H, Katzen F, Beckwith J. Protein disulfide bond formation in prokaryotes. *Annu Rev Biochem*. 2003;72:111-135.

17. Simmons LC, Reilly D, Klimowski L, et al. Expression of full-length immunoglobulins in *Escherichia coli*: rapid and efficient production of aglycosylated antibodies. *J Immunol Methods*. 2002;263:133-147.

18. Venturi M, Seifert C, Hunte C. High level production of functional antibody Fab fragments in an oxidizing bacterial cytoplasm. *J Mol Biol*. 2002;315:1-8.

19. Cereghino GP, Cereghino JL, Ilgen C, Cregg JM. Production of recombinant proteins in fermenter cultures of the yeast *Pichia pastoris. Curr Opin Biotechnol*. 2002;13:329-332.

20. Peterson EC, Farrance C, Henry R, Owens SM. Engineering of a single chain antibody for treatment of methamphetamine abuse. 2004 Experimental Biology meeting abstracts [on CD-ROM]. *FASEB J*. 2004;18:Abstract 397.1.

21. Damasceno LM, Pla I, Chang HJ, et al. An optimized fermentation process for high-level production of a single-chain Fv antibody fragment in *Pichia pastoris. Protein Expr Purif*. 2004;37:18-26.

22. Joosten V, Lokman C, Van Den Hondel CA, Punt PJ. The production of antibody fragments and antibody fusion proteins by yeasts and filamentous fungi. *Microb Cell Fact*. 2003;2:1.

23. Murasugi A, Asami Y, Mera-Kikuchi Y. Production of recombinant human bile salt-stimulated lipase in *Pichia pastoris. Protein Expr Purif*. 2001;23:282-288.

24. Ma JK, Drake PM, Christou P. The production of recombinant pharmaceutical proteins in plants. *Nat Rev Genet*. 2003;4:794-805.

25. Gleba Y, Klimyuk V, Marillonnet S. Magnifection—a new platform for expressing recombinant vaccines in plants. *Vaccine*. 2005;23:2042-2048.

26. Gleba Y, Marillonnet S, Klimyuk V. Engineering viral expression vectors for plants: the 'full virus' and the 'deconstructed virus' strategies. *Curr Opin Plant Biol*. 2004;7:182-188.

27. Marillonnet S, Thoeringer C, Kandzia R, Klimyuk V, Gleba Y. Systemic Agrobacterium tumefaciens-mediated transfection of viral

replicons for efficient transient expression in plants. *Nat Biotechnol*. 2005;23:718-723.

28. Santi L, Giritch A, Roy CJ, et al. Protection conferred by recombinant Yersinia pestis antigens produced by a rapid and highly scalable plant expression system. *Proc Natl Acad Sci USA*. 2006;103:861-866.

29. Gils M, Kandzia R, Marillonnet S, Klimyuk V, Gleba Y. High-yield production of authentic human growth hormone using a plant virus-based expression system. *Plant Biotechnol J*. 2005;3:613-620.

30. Sharp JM, Doran PM. Characterization of monoclonal antibody fragments produced by plant cells. *Biotechnol Bioeng*. 2001;73:338-346.

31. Derouazi M, Girard P, Van Tilborgh F, et al. Serum-free large-scale transient transfection of CHO cells. *Biotechnol Bioeng*. 2004;87:537-545.

32. Baldi L, Muller N, Picasso S, et al. Transient gene expression in suspension HEK-293 cells: application to large-scale protein production. *Biotechnol Prog*. 2005;21:148-153.